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Effects of Frangipani Flower Extract (*Plumeria acuminata* L.) Against the Mortality of *Aedes aegypti* Larvae

Eka Lidya[1], Prayudhy Yushananta[1*]

[1] Politeknik Kesehatan Kementerian Kesehatan Tanjung Karang, Lampung. Indonesia

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Abstract

Bioinsecticide development has become a demand after the adverse effects of long-term use of synthetic chemical insecticides, including vector resistance and environmental pollution. In particular, this study investigated the ability of Frangipani flower extract (*Plumeria acuminata* L.) as larvacidal against *Aedes aegypti* instar III. The study used a Factorial Completely Randomized Design (two replications), conducted on March-May 2023. Plant extraction by maceration using 96% ethanol solvent. Bioassay tests were carried out at three concentration levels (5%, 7%, 10%) and four levels of contact time (6, 12, 18, 24 hours), with 25 larvae in each treatment. Spectrophotometry methods measured flavonoid and saponin content. The two-way ANOVA test analyzed data, the Tukey test, and the probit test to obtain LC_{50} and LC_{90}. The study found flavonoid and saponin content of 4.43 Mg QE/g and 2.45%, respectively. The lowest total mortality (24 hours after exposure) of Aedes aegypti larvae was at a concentration of 5% (MR= 58%) and highest at concentrations of 7% and 10% (MR= 100%). The 7% concentration is the best performance as a larvicide. Statistical analysis showed differences in mortality based on concentration (P = 0.0001) and contact time (P = 0.0001). Mortality of larvae was caused by damage to the respiratory and digestive systems, as seen from the lateral hair, siphon, and abdomen condition. The results of the probit test obtained LC_{50} and LC_{90} of 4.85% (4.36–5.10%) and 5.86% (5.49-7.20%), respectively. The study has proven that *Plumeria acuminata* L. flower extract has the potential as a biolarvaside against *Aedes aegypti*, with a mortality effect reaching 100% after 24 hours of exposure.

Pengembangan bioinsektisida menjadi tuntutan setelah dampak buruk penggunaan insektisida kimia sintetis dalam jangka panjang, seperti resistensi vektor dan pencemaran lingkungan. Secara khusus, penelitian bertujuan menyelidiki kemampuan ekstrak bunga cja (*Plumeria acuminata* L.) sebagai larvasida terhadap *Aedes aegypti* instar III. Penelitian ini menggunakan Rancangan Acak Lengkap Faktorial (dua kali ulangan), dilakukan pada bulan Maret-Mei 2023. Ekstraksi tanaman dilakukan dengan cara maserasi menggunakan pelarut etanol 96%. Uji bioasai dilakukan pada tiga level konsentrasi (5%, 7%, 10%) dan empat level waktu kontak (6, 12, 18, 24 jam), dengan 25 larva pada setiap perlakuan. Pengukuran kandungan flavonoid dan saponin dengan metode spektrofotometri. Keseluruhan data dianalisis dengan Uji ANOVA two way, uji Tukey, serta uji probit untuk memperoleh LC_{50} dan LC_{90}. Kadar flavonoid dan saponin masing-masing sebesar 4,43 Mg QE/g dan 2,45%. Mortalitas larva *Aedes aegypti* terendah (24 jam setelah paparan) terdapat pada konsentrasi 5% (MR= 58%) dan tertinggi pada konsentrasi 7% dan 10% (MR= 100%). Konsentrasi 7% menunjukkan hasil terbaik dari larvasida. Hasil analisis statistik menunjukkan perbedaan mortalitas berdasarkan konsentrasi (P = 0,0001) dan waktu kontak (P = 0,0001). Mortalitas larva disebabkan oleh kerusakan pada sistem pernapasan dan pencernaan, terlihat dari kondisi rambut lateral, sifon, dan abdomen. Hasil uji probit diperoleh LC_{50} dan LC_{90} masing-masing sebesar 4,85% (4,36–5,10%) dan 5,86% (5,49–7,20%). Penelitian ini membuktikan bahwa ekstrak bunga *Plumeria acuminata* L. berpotensi sebagai biolarvasida terhadap *Aedes aegypti*, dengan efek mortalitas mencapai 100% setelah 24 jam paparan.

* Corresponding author: Prayudhy Yushananta
Politeknik Kesehatan Kementerian Kesehatan Tanjung Karang, Lampung. Indonesia
Email: prayudhyyushananta@gmail.com

INTRODUCTION

Mosquitoes are vectors responsible for the spread of several dangerous diseases that can cause death, including dengue fever. The dengue virus causes dengue fever and is mainly transmitted by mosquitoes of the species *Aedes aegypti* Linnaeus. 1762 (Diptera: Culicidae) (Amutha et al., 2019; Kumar et al., 2020). Each year, dengue fever infects around 230 million people, and 40,000 ends in death (WHO, 2020; Yushananta, 2021).

Mosquito control is the primary method in preventing the spread of disease, and control at the larval stage is the most effective strategy (Bilal et al., 2017; Duguma et al., 2017). The use of synthetic chemical insecticides has been the choice for the past five decades (Duguma et al., 2017). The continuous and widespread use of synthetic chemical insecticides has caused vector resistance, environmental pollution affected the nutrient cycle in primary production, and death in non-target insects (Bej et al., 2021; Kamran et al., 2022; Khan, 2022; Kim et al., 2021; Schlechtriem et al., 2012; Silalahi et al., 2022; Smith et al., 2016; Wang et al., 2017). Natural insecticides are the best choice to overcome losses due to the use of synthetic chemical insecticides because they are environmentally safe, readily biodegradable, low cost, and abundant raw materials (Benelli & Beier, 2017; Bilal et al., 2017; Pavela et al., 2019; Senthil-Nathan, 2020; Yushananta & Ahyanti, 2021).

One of the plants that can be developed as a larvicide is the Frangipani flower (*Plumeria acuminata* Linnaeus), widespread throughout Indonesia. In traditional medicine systems, this plant is widely used for various treatments (as a laxative, diarrhea medicine, itching medicine, anti-rheumatic, anti-inflammatory, and antioxidant) because it contains flavonoid, terpenoid, tannin and a little saponin compounds (Adnyani Suari et al., 2021; Attasih et al., 2024; Rutuba et al., 2021; Sari et al., 2023; Shofi et al., 2020; Taid et al., 2016; Zaheer et al., 2010). Bioactive compounds (including alkaloids, saponins, tannins, and flavonoids) can also be used as larvicides because they cause death through digestion, respiration and the nervous system (Chaieb, 2017; Govindarajan et al., 2016; Hidayati & Suprihatini, 2020; Pavela et al., 2019; Rohmah et al., 2020; Yushananta & Ahyanti, 2021).

To our knowledge, there is no report that specifically measures the content of bioactive compounds (flavonoids and saponins) of *Plumeria acuminata*, and assesses their ability as larvicides against *Aedes aegypti*. In the present study, the flower extract of *Plumeria acuminata* Linnaeus. 1753 (Gentianales: Apocynaceae) was measured for flavonoid and saponin content and then analyzed for larvicidal ability against the *Aedes aegypti*.

METHODS

Study design and setting

The study used a factorial Completely Randomized Design (CRD) with two replications. The variables studied were concentrations of three levels (5%, 7%, and 10%) and contact time (6, 12, 18, 24 hours). Plant samples were collected from Bandar Lampung City (South Latitude 5°21'15", West Longitude 105°13'01"E, altitude 124 meters). The study was conducted from March to May 2023 at the Tanjungkarang Health Polytechnic Laboratory and the Baturaja Public Health Laboratory, South Sumatra. The study has obtained ethical approval from the Tanjungkarang Health Polytechnic Health Ethics Commission, Number 162/KEPK-TJK/II/2023.

Plant extraction

Plumeria acuminata L flowers (local name is kamboja) that were collected were washed repeatedly with distilled water to remove dirt and dried. Four hundred grams of dried flowers were ground and soaked in 500 mL of 96% ethanol for 24 hours in a closed container, then filtered to separate the filtrate and residue. The residue was dried from the solvent and repeated thrice with the same treatment. The entire filtrate was evaporated with a rotary evaporator at a temperature 40-60 ℃ until a concentrated extract (100% concentration) was obtained. A total of 20 mL of the extract was tested for saponin and flavonoid content using the spectrophotometry method.

Rearing Aedes aegypti larvae and Bioassay-test

The rearing process is carried out to obtain the original gene *Aedes aegypti* larvae. The initial stage is making a hatching medium using terabit and water (2 cm high) into a tray, then stirring and covering with gauze for 1x24 hours. The eggs are

put into the tray the next day and then covered with gauze. In the hatching process, feeding was carried out twice (morning and evening) using dog food, and the cleanliness and clarity of the water were checked. The rearing process followed Kauffman et al. (2017).

Following WHO (2005), the bioassay test used instar-III larvae because they were already large enough to be easily identified. Each treatment used 25 larvae and was equipped with positive and negative controls totaling 25 larvae. The total of *Aedes aegypti* larvae used for the study was 1,800.

A total of 25 instar-III *Aedes aegypti* larvae were put into a test container and exposed to extract at concentrations of 5%, 7%, and 10%. Following WHO (2005), the extract was diluted using distilled water. The positive and negative controls only used 100 ml of distilled water. The treatment was carried out with two repetitions and random in each experiment. According to WHO (2005), larval mortality is determined based on visual and larval conditions, namely sinking to the bottom of the container, not moving, and not responding to stimuli in the form of touch using a stick on the siphon. Mortality recording is carried out on each sample.

Data Analysis

Data analysis was conducted using the Two-way ANOVA test to determine the difference in Aedes aegypti larval mortality based on research variables (concentration and contact time). The Tukey test was also applied to obtain valid differences between treatment levels. In this study, a probit analysis was also carried out to obtain the LC_{50} value (effective concentration against 50% death of *Aedes aegypti* larvae) and LC_{90} (effective concentration against 90% death of *Aedes aegypti* larvae). All data were analyzed with SPSS 24.0 at a 95% confidence level.

RESULTS

The results of the flavonoid content test of the *Plumeria acuminata* L. flower extract is 4.43 Mg QE/g sample, meaning that the total flavonoids from every 1 gram of extract are equivalent to 4.43 mg of quercetin. The saponin content is 2.45% of every 1 gram of extract.

The study results (Table 2) obtained the average larval mortality (two replications) at a 5% extract concentration of 6.75 (1-20), a 7% concentration of 15.75 (5-25), and 10% of 15.25 (5-25). In the control, there was no larval mortality.

Table 1. Flavonoid and saponin content

	Flavonoid (Mg QE/g)	Saponin (%)
Replication 1	4.43	2.47
Replication 2	4.46	2.45
Replication 3	4.40	2.43
Average	4.43	2.45

Larval mortality was observed for 24 hours of exposure, with four recordings (6, 12, 18, 24 hours). The average larval mortality was 3.83 (1-6) after six hours of exposure, 9.17 (2-13) after 12 hours of exposure, 15.83 (7-21) after 18 hours of exposure, and 21.50 (9-25) after 24 hours of exposure. The percentage of larval mortality (mortality rate/MR) after 24 hours of exposure at concentrations of 5%, 7%, and 10% were 58%, 100%, and 100%, respectively.

Table 2. Mortality of *Aedes aegypti* larvae based on concentration and contact time

Concentration	6 hours		12 hours		18 hours		24 hours		Average	MR (%)
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2		
5%	1	1	2	5	7	9	9	20	6.75	58.0
7%	6	5	12	12	20	21	25	25	15.75	100.0
10%	5	5	13	11	21	17	25	25	15.25	100.0
Control	0	0	0	0	0	0	0	0	0	
Average	3.83		9.17		15.83		21.50		12.58	

R= Replication; MR= Mortality Rate

Figure 1 shows that the lowest larval mortality was at a concentration of 5%, one larval after one hour of exposure, and 14.50 after 24 hours of exposure. At a concentration of 7%, larval mortality was 5.00 one hour after exposure and 25.00 after 24 hours of exposure. While at a concentration of 10%, larval mortality was 5.50 one hour after exposure and 25.00 after 24 hours of exposure. Figure 1 also shows that larval mortality follows the rate of contact time. The longer the exposure, the higher the mortality of larvae.

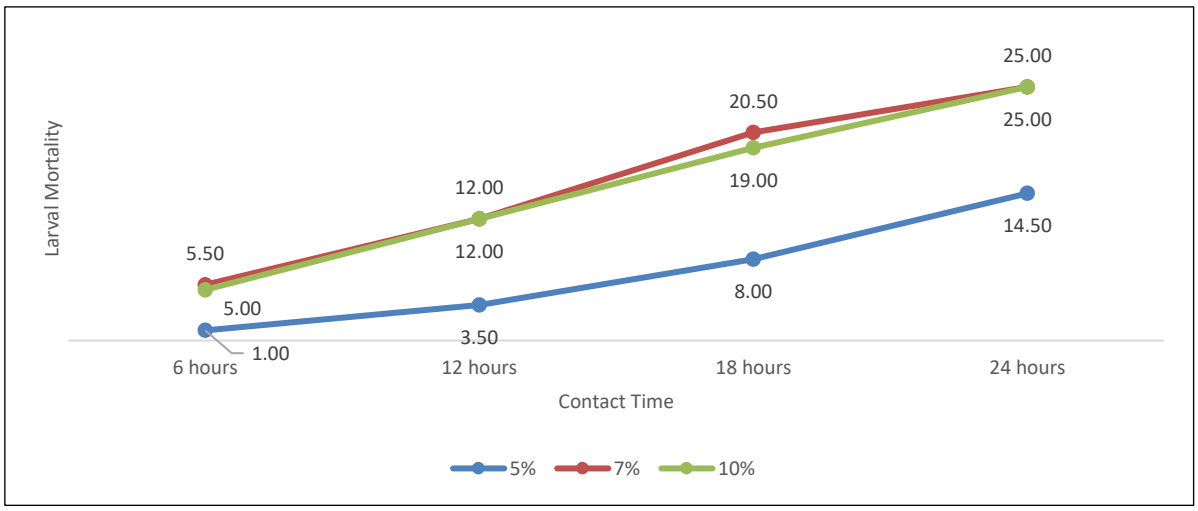

Figure 1. Larval mortality based on concentration and contact time

Effect of concentration and contact time

The Two-way ANOVA test was used to validly assess the differences in larval mortality based on concentration and contact time. Furthermore, the Tukey test was applied to determine the difference in larval mortality based on the treatment level.

The analysis results (Table 3) showed differences larval mortality based on the concentration of *Plumeria acuminata* L. extract (F = 31.49; P = 0.0001). The Tukey test (Table 4) explained the differences in larval mortality at a concentration of 5% compared to 7% and 10%. Meanwhile, there was no significant difference between the concentrations of 7% (mean = 15.75) and 10% (mean = 15.25). It can be seen that the 7% concentration showed the best performance as a larvicide.

Table 3 also shows significant differences larval mortality based on variations in contact time (F = 54.86; P = 0.0001). Differences in larval mortality are shown at each level of contact time.

Table 3. The Two-way ANOVA test result

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	1523.83	11	138.53	21.31	0.0001
Intercept	3800.17	1	3800.17	584.64	0.0001
Concentration	409.33	2	204.67	31.49	0.0001
Contact time	1069.83	3	356.61	54.86	0.0001
Concentration * Contact time	44.67	6	7.44	1.15	0.395
Error	78.00	12	6.50		
Total	5402.00	24			
Corrected Total	1601.833	23			

a. R Squared = 0.951 (Adjusted R Squared = 0.907)

Table 4. Tukey test results

Concentration	Subset	
	1	2
5%	6.75	
7%		15.75
10%		15.25
P	1.000	0.919

Contact Time	Subset			
	1	2	3	4
6 hours	3.83			
12 hours		9.17		
18 hours			15.83	
24 hours				21.50
P	1.000	1.000	1.000	1.000

Lethal concentration

Probit analysis was performed to obtain the LC_{50} value (effective concentration against 50% death of *Aedes aegypti* larvae) and LC_{90} (effective concentration against 90% death of *Aedes aegypti* larvae). Probit analysis results get an LC_{50} value of 4.85% (4.36 – 5.10%), while LC_{90} is 5.86% (5.49 – 7.20%).

DISCUSSION

The study results showed that *Plumeria acuminata* L. flower extract has high bioactive flavonoid and saponin compounds, were 4.43 ± 0.03 mg QE / g and 2.45 ± 0.02% of each gram of sample. Meanwhile, according to Taid et al. (2016), the flavonoid content of the leaves was 0.201 ± 8.41 mg QE/g. These results indicate that the flavonoids in the flower are higher than in the leaf. However, the flavonoid content in Plumeria acuminata L. flowers is smaller than the flavonoid content in *Plumeria pudica* Jacq. (Gentianales: Apocynaceae) leaf extract, which is 108.33 ± 1.67 mg QE/g (Rutuba et al., 2021). Likewise, in *Plumeria alba* L. leaves (Gentianales: Apocynaceae), it was 29.73 ± 6.74 mg QE/g (Attasih et al., 2024).

Only a few studies have analyzed the flavonoid content in Plumeria sp. quantitatively. Most studies report flavonoid content through qualitative tests, as reported by Shofi et al. (2020), Utami & Cahyati (2017), Nuryanti & Haryoto, (2023), Fikayuniar et al. (2023), Adnyani Suari et al. (2021), Sura et al. (2018), and Sari et al. (2023).

Bioactive compounds in plant extracts, such as alkaloids, flavonoids, saponins, and tannins, are essential compounds in plant chemical defenses constitutively or induced against microorganisms, such as fungi, bacteria, viruses, and harmful insects (Pavela et al., 2019). Alkaloid, saponin, tannin and flavonoid compounds in plants can cause death to larvae through digestion, respiration, and the nervous system (Ahyanti et al., 2022, 2023; Ahyanti & Yushananta, 2023a, 2023b; Chaieb, 2017; Govindarajan et al., 2016; Hidayati & Suprihatini, 2020; Pavela et al., 2019; Pratama & Yushananta, 2021; Putri & Yushananta, 2022; Rohmah et al., 2020; Yushananta & Ahyanti, 2021). Figure 2 shows siphon and digestive damage in larvae.

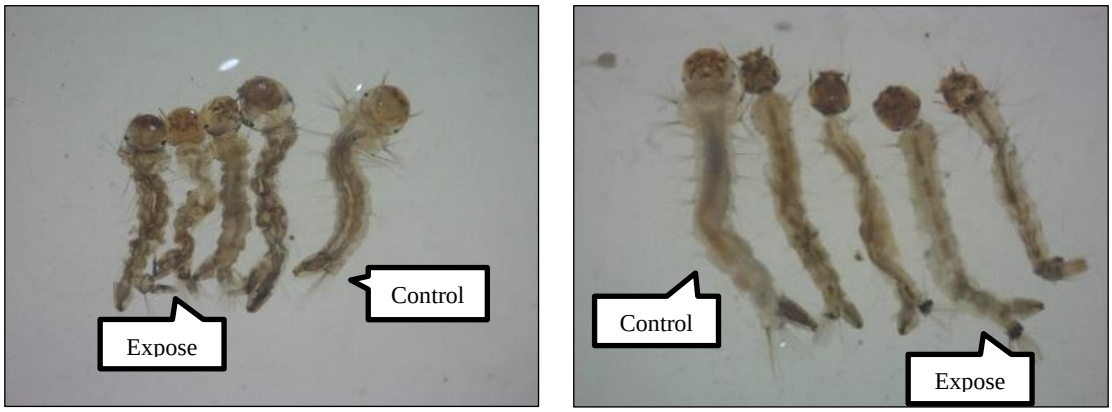

Figure 2. Damage to the lateral hair, siphon, and abdomen of larvae

The study results (Table 2) obtained the highest average larval mortality at a concentration of 7% of 15.75 (5-25), and the lowest at a concentration of 5% extract was 6.75 (1-20). Based on the contact time (Figure 1), the lowest larval mortality was at a concentration of 5%, which was 14.50 after 24 hours of exposure (larval mortality rate = 58.0%). The highest concentrations were at 7% and 10%, with a larval mortality rate reaching 100%. These results show no significant difference in mortality between concentrations of 7% and 10% (Table 4). Thus, using both concentrations provides the same larvicide effectiveness against *Aedes aegypti*. Figure 1 also shows that larval mortality follows the rate of exposure time. The longer the exposure, the higher the mortality of larvae.

The concentration aligns with the amount of bioactive compound content in biolarvacides. The higher the concentration, the higher the content of bioactive compounds (Adnyani Suari et al., 2021; Ahyanti et al., 2023; Ahyanti & Yushananta, 2022, 2023b; Juariah & Irawan, 2017; Nasution & Ulina, 2022; Nikmah et al., 2016; Ningsi et al., 2016; Pratama & Yushananta, 2021; Putri & Yushananta, 2022; Utami & Cahyati, 2017).

Many studies have reported a positive relationship between exposure and larval mortality. The longer the larvae are exposed, the more toxic compounds (bioactive) enter their bodies, causing damage to their digestive, respiratory, and nervous systems (Ahyanti et al., 2023; Ahyanti & Yushananta, 2022, 2023b; Juariah & Irawan, 2017; Nasution & Ulina, 2022; Ningsi et al., 2016; Pratama & Yushananta, 2021; Putri & Yushananta, 2022).

The mechanism of damage caused by bioactive compounds to larvae resulting in death are: 1) bioactive compounds that enter through the siphon will cause damage to the siphon so that the larvae cannot breathe; 2). bioactive compounds that enter the digestive tract will inhibit electron transport in the mitochondria so that the formation of energy from food as an energy source in cells does not occur and cells cannot be active; 3) bioactive compounds can form protein complexes and damage cell membranes by denaturing protein bonds in the cell membrane so that the cell membrane becomes lysed (Ahdiyah & Purwani, 2015; Ahmad & Adriyanto, 2019; Juariah & Irawan, 2017; Pavela et al., 2019). In addition, according to Chaieb (2017), bioactive compounds will affect the work of the larval cholinesterase enzyme, causing disorders in the respiratory, digestive, and motor systems. Figure 2 proves damage to larvae due to exposure to *Plumeria acuminata* L. flower extract.

The results of the analysis (Table 3) show differences in *Aedes aegypti* mortality based on variations in the concentration of *Plumeria acuminata* L. flower extract ($F = 31.49$; $P = 0.0001$) and contact time ($F = 54.86$; $P = 0.0001$). The Tukey test (Table 4) shows differences in larval mortality at 5% between 7% and 10%. Meanwhile, between the concentrations of 7% and 10% did not show a significant difference. The difference in larval mortality was shown at each level of contact time (Table 4). The lowest mortality was six hours after exposure, and the highest was 24 hours after exposure. The positive relationship between concentration and contact time at larval mortality has been explained in many studies. Concentration and contact time are related to the quantity of exposure by bioactive compounds to larvae.

The study results obtained LC_{50} and LC_{90} of 4.85% (4.36–5.10%) and 5.86% (5.49-7.20%) respectively. The results of this study are lower than the findings of (Utami & Cahyati, 2017) which obtained LC_{50} and LC_{90} of 9.041% and 26.774% respectively. Meanwhile, Sura et al. (2018) obtained an LC_{50} of 0.0218% in *Plumeria alba* L. flower extract.

Several studies have also reported LC*50* in the use of biolarvacides, including *Piper betle* leaves of 5.556% (Adibah & Dharmana, 2017), *Artocarpus altilis* flowers of 70% (Nikmah et al., 2016), *Carica papaya* leaves of 3.73% and 7.55% (Ramayanti & Febriani, 2016), *Citrus x aurantium* peel 0.20% (Widyasari et al., 2018), *Pandanus amaryllifolius* leaves 9.445% (Kasma et al., 2019), *Annona muricata* leaves 0.736% (Kewa et al., 2020), *Eagle mermelos* leaves 4.12% and 10.82% (Puspa Sari & Priastini Susilowati, 2019). Based on the results of these studies, the *Plumeria acuminata* L. flowers extract has the potential as a larvicide against *Aedes aegypti*.

CONCLUSION

The study has proven that *Plumeria acuminata* L. flower extract has potential as a larvicide against *Aedes aegypti* instar III. The lowest mortality was at a concentration of 5% and six hours of exposure. Mortality reached 100% at concentrations of 7%

and 100% after 24 hours of exposure. The statistical analysis proved the effect of concentration (P=0.0001) and contact time (P=0.0001) on larval mortality. The 7% concentration showed the best performance as a larvicide. Mortality of larvae was caused by damage to the respiratory and digestive systems, as seen in lateral hair, siphon, and abdomen. The probit test results obtained LC_{50} and LC_{90} of 4.85% (4.36–5.10%) and 5.86% (5.49-7.20%), respectively.

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